Exhibit C

Post-Implantation Alterations of Polypropylene in the Human

Gina Sternschuss,* Donald R. Ostergard and Hiren Patel

From the Long Beach Memorial Medical Center, Long Beach, California, and University of Louisville (DRO), Louisville, Kentucky

Purpose: We reviewed the mechanisms by which polypropylene mesh changes after implantation in the human body.

Materials and Methods: The existing polymer and medical literature was reviewed regarding polypropylene, including its chemical characteristics, and compositional and physical properties, which undergo alteration after implantation at various human body locations. We also reviewed the changes in those physical properties that were demonstrable in explanted specimens.

Results: Polypropylene in mesh form is commonly considered inert and without adverse reactions after implantation in humans. The literature suggests otherwise with reports of various degrees of degradation, including depolymerization, cross-linking, oxidative degradation by free radicals, additive leaching, hydrolysis, stress cracking and mesh shrinkage along with infection, chronic inflammation and the stimulation of sclerosis. Many substances added to polypropylene for various purposes during manufacture behave as toxic substances that are released during the degradation process. The material may also absorb various substances. These alterations in the chemical structure of polypropylene are responsible for visibly demonstrable fiber changes, resulting in the loss of structural integrity through material embrittlement. The heat of manufacturing polypropylene fibers begins the degradation process, which is augmented by the post-production heat used to flatten the mesh to prevent curling and attach anchoring appendages.

Conclusion: Based on available evidence the polypropylene used for surgical treatment of various structural defects is not inert after implantation in the human body. The quest for the perfect mesh must continue.

Key Words: polypropylene, surgical mesh, materials testing, toxicity, complications

Abbreviations and Acronyms

DSC = differential scanning calorimetry

PP = polypropylene

SEM = scanning electron microscopy

Submitted for publication May 18, 2011.

* Correspondence: 701 East 28th St., Suite 212, Long Beach, California 90806 (telephone: 562-426-5630; FAX: 562-492-9893; e-mail: docgina2000@yahoo.com).

ACCORDING to the Technical Committee 150 on implants for surgery of the International Organization for Standardization implants are defined as "objects or devices which are surgically placed in the body either temporarily or permanently for diagnostic or therapeutic purposes."[1] PP is such a permanently implanted biomaterial. It is used in the form of a mesh to replace and restore the function of destroyed or damaged tissue in the human body. It is continuously in contact with human bodily fluids and subject to their effects.[2]

A biomaterial must show biocompatibility and be inert without adverse reactions in the body. Biocompatibility should be viewed not as a specific property of a material, but rather as a dynamic process that allows it to perform a function in the body and not just reside there. This function directly depends on the material-tissue interaction. The material must be nontoxic, noncarcinogenic and

0022-5347/12/1881-0027/0
THE JOURNAL OF UROLOGY®

Vol. 188, 27-32, July 2012
Printed in U.S.A.
DOI:10.1016/j.juro.2012.02.2559

resistant to degradation, have acceptable strength, be able to withstand sterilization while being flexible (able to be formed into different shapes and sizes), and be relatively low cost.[2]

Innovations in each surgical subspecialty are accompanied by the development of new techniques and materials. Surgeons rarely question the genesis of the different properties of biomaterials, much less have an interest in variations in chemical formulation, molecular weight and other properties or methods of production and sterilization, and their effects on a particular mesh. Certainly surgeons have a reasonable expectation that the manufacturer of a kit has sufficiently researched its usefulness and claims can be substantiated.

Even with this expected background none of the suppliers of the new meshes publicize the basic concept that there is no such thing as a completely inert implant, material or device.[1] Human tissue may tolerate or reject the implanted material, as the surgeon inevitably experiences when patients return with extruded mesh.

POLYPROPYLENE HISTORY

PP was first polymerized in 1954 by Rehn and Natta.[3] In 1960 PP mesh was first introduced by Usher et al for hernia repair.[4] Since then, new and improved meshes have been introduced and widely used in various surgical subspecialties, mainly to repair weak tissue in the anterior abdominal wall under completely sterile conditions. Groups searched for the material that, if found, could "artificially produce tissue of density and toughness of fascia and tendon" so that "the secret of radical cure for hernia would be discovered."[5]

POLYPROPYLENE CHEMICAL CHARACTERISTICS

Petroleum refining produces various byproducts and one of them is PP, which is made chemically by polymerizing propylene. The actual chemical reaction requires specific heat and pressure conditions, and a catalyst. The polymerization process results in a chain representing the PP molecule, which may contain thousands of propylene molecules. The melting point of various PP forms is between 320F and 340F.[6] Such extremes of heat are used after manufacture to prevent mesh folding and rolling, and to apply the anchoring devices used in some kits. The mesh is hydrophobic and nonhydrolyzable.

During the manufacturing process various stabilizers are added immediately after production to maintain the desired physical characteristics of the resulting mesh. These stabilizers are in large part responsible for the tissue responses observed after implantation since they may diffuse from the polymer into tissue and cause a tissue reaction.[3] We provide a partial list of the additives that can be added to PP.[6] Generally we have no idea of exactly what was used in a particular mesh construct. There is no such thing as pure PP for use in medicine.

After implantation PP mesh absorbs certain substances from bodily fluids, notably cholesterol and fatty acids, which could alter the physical and mechanical properties of the mesh.[7]

POLYPROPYLENE STERILIZATION

Autoclave heating or γ radiation is used for sterilization. Each process may potentially alter the molecular structure of PP by creating cross-links (attachment) of 1 PP chain to another or fracturing the chains to produce smaller chains.[8] These reactions may be responsible for alterations in the mesh, which may ultimately lead to deep cracking of the mesh surface and subsequent mesh failure. Repetitive gas or heat sterilization causes mesh shrinkage with an alteration in mechanical strength, decreasing the usefulness of mesh for tissue repair.[8]

Exposure to γ radiation increases PP susceptibility to oxidation and causes oxidation.[6,9] Free radicals formed during γ radiation sterilization become trapped in the crystalline structure of the polymeric material and disrupt its molecular structure. The resulting cross-linking and chain scission (chain breaking) lead to increased embrittlement of the material.

Generally PP is highly susceptible to oxidation due to its chemical structure. Oxidation occurs through a chain reaction involving free radicals, which in turn produces hydroperoxide, which produces more free radicals to continue the oxidative process. The presence of oxygen, heat or mechanical stress causes changes in the molecular structure of PP, leading to a change in its mechanical properties.[6]

POLYPROPYLENE PHYSICAL CHARACTERISTICS

PP is preferentially used to produce fibers due to its melting characteristics and low density. There are monofilament and multifilament fiber types. Monofilaments are ribbons of a single strand of PP that are applied to weave currently used meshes. They have high resistance to deformation and good strength, retain strength when wet, do not absorb water and have high stiffness and good tensile strength.[6] Multifilament fibers are formed by gathering several individual filaments in a bundle, which are twisted to stay together during weaving. The interfiber distance is small and so they are more difficult to clean

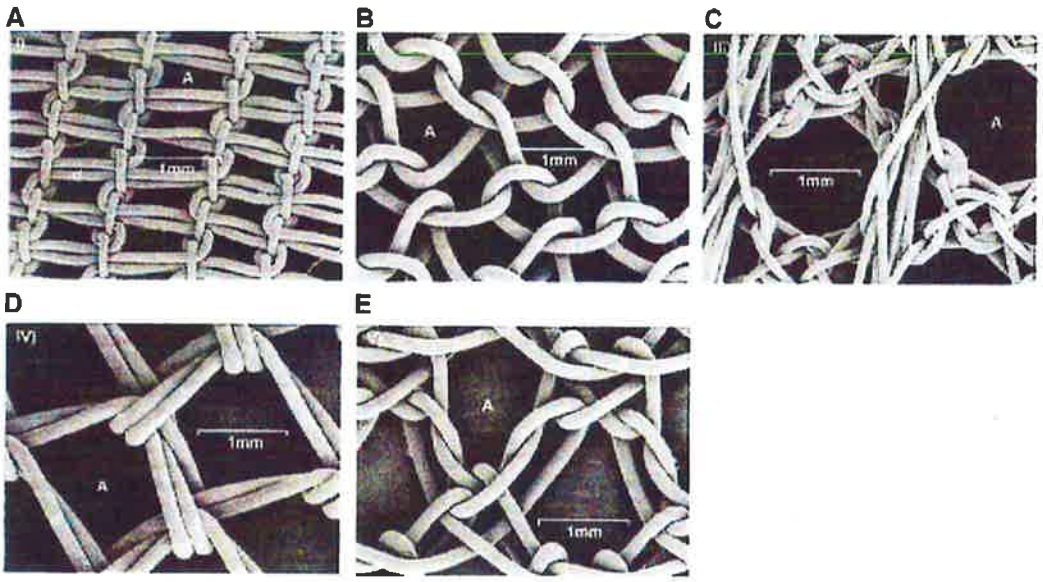

Figure 1. Varying complexity of differing PP weave types varies total surface area exposed to body microenvironments. *A*, Aris®. *B*, AutoSuture®. *C*, Avaulta®. *D*, TVT™. *E*, Uretex®. Scale bars indicate 1 mm. Reprinted with permission.[21]

since small particles are easily captured between the filaments.[6]

The physical properties of the biomaterial are of great importance, including filament type and diameter, the cumulative surface area of a given mesh product, surface characteristics of the fiber (smooth vs rough), pore size, weight, flexibility, porosity and weave type (knitted vs woven). These characteristics vary widely among currently manufactured meshes (fig. 1). To our knowledge the combination of characteristics that is critical for optimal performance is currently unknown.

The categories of Amid were based on mesh use for hernia repair in a completely sterile surgical setting.[5] There are 4 categories by pore size, including macroporous (pores larger than 75 μ), microporous (pores less than 10 μ) macroporous mixed with microporous or with multifilamentous components and subμ pore size. It is currently unknown whether this classification is germane to mesh implantation in the vagina since the vagina is considered clean contaminated in nature due to the inability to completely sterilize it.[10]

POLYPROPYLENE IN HUMAN BODY

Toxicity

In pristine form PP has no toxicity and is compatible with tissue. However, numerous substances are added to it and these extractable materials produce toxic reactions.[11] Toxicity depends on the extent of the migration of toxic materials into the body after implantation. The migration of a toxic substance also depends on the chemical properties of the toxic substance, including molecular weight, polarity and solubility.

Substances on the material surface more readily migrate into the extracellular bodily fluids around them. The diffusion process is increased by heat sterilization of the material, which leads to migration of the toxic substances to the surface of the polymer. In addition to various volatile organic compounds, certain substances, generally stabilizers, that are added to or present in PP mesh are potentially toxic and available for release into the body, including plasticizers (to increase plasticity), primary antioxidants (phenolics, aromatic amines and butylated hydroxytoluene), secondary antioxidants (peroxide decomposers and phosphites), ultraviolet and radiation stabilizers (for absorption with heat release), acid scavengers (antacids), free radical scavengers (to stabilize free radicals), anti-ozonants (to counteract ozone), residual monomers (left over from chain formation), catalysts, nucleating agents (which affect mechanical properties and processing), antistatic agents (to prevent dust adherence), colorants (pigments or dyes), and antiblocking and slip agents (to prevent sheets from sticking together).[6]

After PP exposure to high temperature Frostling et al identified some toxic substances that interfered with renal, hepatic and pulmonary function in animals.[12] It was not possible to measure these toxic products in vivo but they noted PP degradation changes in vitro at temperatures as low as 25C. Changes have also been noted in the myocardium along with decreased ability to produce antibodies. In addition, there are reports of pathomorphological

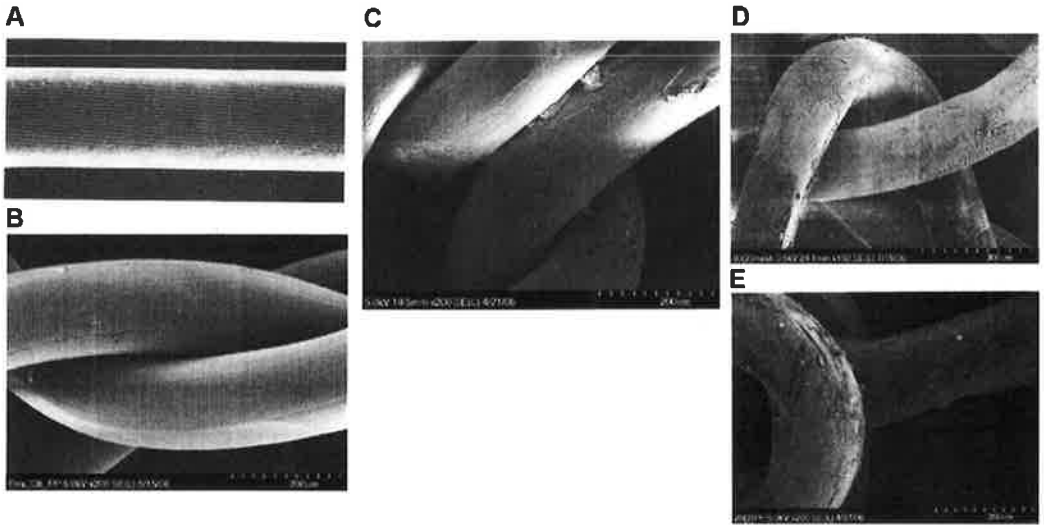

Figure 2. Pristine PP and progressive degradation. *A*, smooth surface without surface grooving that would increase surface area. Reprinted with permission.[6] *B*, this fiber is considered pristine but grooves and striations due to manufacturing process begin degradation. Scale bar indicates 200 μm. *C*, further degradation with peeling of fiber surface. Scale bar indicates 200 μm. *D*, transverse cracking in fibers, which are partially peeled. Scale bar indicates 300 μm. *E*, deep grooving and blisters on fibers. Scale bar indicates 200 μm. Reprinted with permission.[16]

changes in the liver and kidneys of mice after PP administration.[13] To our knowledge it is unknown whether these changes also occur in humans.

Degradation

Immediately upon insertion an acute inflammatory reaction begins. Neutrophils are the first cells to arrive. They begin to produce oxidants, including hydrogen peroxide and hypochlorous acid, which continue the heat induced oxidative process begun during manufacture.[14] In the environment of the human body polymers undergo varying degrees of degradation.[15] Oxidation of the PP chains produces free radicals, which cause various events, including depolymerization (breakdown of the molecular chain), cross-linking, oxidative degradation, additive leaching (which may include toxic substances), hydrolysis and stress cracking. This process produces more free radicals, which perpetuate the chain reaction. Enzyme catalysts allow these reactions to occur at body temperature.[13,14] Ultimately due to the breakdown of the structural integrity of the PP polymer chains the mesh shows signs of surface alterations initially and then deep cracking of the fibers. The end result is lessened structural integrity with changes in molecular weight and crystallinity. The mechanical integrity of the implanted mesh is also decreased.

Enzymes released from traumatized cells in the post-implantation period are responsible for this degradative process, which is nonlinear with time.[13] The implant may be more susceptible to the enzymes such as myeloperoxidase that are produced in the acute inflammatory phase and less so to those produced during the chronic inflammatory period or vice versa.[13]

To determine the effects of the body on implanted mesh, which are similar to changes after transvaginal mesh implantation,[7] explanted PP hernia mesh samples were compared to intact mesh samples.[16] After the samples were cleaned, they were tested by SEM, DSC (which measures absorbed or produced energy by heat application) and thermogravimetric analysis (which measures weight change in relation to temperature), followed by compliance testing. Before implantation SEM has shown that intact mesh fibers are smooth with no evidence of damage, degradation or cracking (fig. 2, *A*).[6] However, SEM has also revealed linear striations and grooving, which increase the surface area and are likely the result of heat and other mechanical factors used in the manufacturing process, such as extrusion, which start the degradation process (fig. 2, *B*).[8] The explanted mesh samples appeared rough with peeling fibers and transverse or longitudinal cracks and fissures as well as blisters on the fiber surface.[16] DSC revealed a lower melting temperature and thermogravimetric analysis showed a shift to higher peak temperature as a result of degradation (fig. 2, *C* to *E*).[16] Compliance testing revealed 4 to 30 times decreased compliance. This study showed severe oxidative degradation of PP mesh in vivo.

Various factors may affect the rate and severity of polymeric implant oxidation, including implantation duration in the body and potentially some patient related factors, such as age, smoking, diabetes and

body mass index.[16] Patients also have variable reactions to implanted mesh depending on whether the patient is a high responder, which produces an exaggerated tissue reaction. To our knowledge there is no good way to determine the anticipated response of an individual, although increases in tumor necrosis factor, cytokines and interferon are associated with adverse events. In a study of various types of explanted polymeric materials inflammatory reaction cells were present on the tissue-mesh explants up to 8 years after implantation.[17]

Other researchers also observed PP degradation after human implantation. The ophthalmology literature provides examples of the degradation process that show changes similar to those in explanted transvaginal mesh samples.[7] In a study by Jongebloed and Worst of a suture from the eye, which was removed 6.5 years after implantation, SEM revealed longitudinal cracks.[18] Part of the surface layer was missing, the diameter toward the end was decreased more than 50% and the subsurface layer had a fibrillar structure with deep surface cracking. This degradation process is considered to be caused by the action of oxidative enzymes on the PP suture. Manufacturing grooves were noted in the sample considered to be pristine. In a subsequent study by Jongebloed et al SEM revealed severe degradation after only 1 year of exposure to bodily fluids.[19] Altman et al, who commented that ultraviolet exposure was a likely contributing factor, also observed severe degradation on SEM.[20] All meshes were implanted under sterile conditions.

Clavé et al described 100 implants of PP and polyethylene terephthalate prostheses that were removed from patients treated with prior pelvic floor prolapse repair.[7] This was the first report of degradation in transvaginally implanted mesh. The materials were removed due to complications. SEM, Fourier transform infrared spectrometry and DSC were used to analyze the samples, which were compared to nonimplanted, intact materials analyzed in the same fashion. Analysis of explants revealed material degradation, deep surface cracks, flaking, detachment of the material and peeling of the fibers. Various tissue reactions were also noted, such as infection, chronic inflammation and fibrosis. Of the monofilament meshes 33.3% were degraded. More degradation was noted in the presence of acute infection or chronic inflammation. All mesh implants had been placed in the clean contaminated environment of the vagina. Figure 3 shows an example of degraded mesh on SEM.

Such degraded mesh produces undesirable consequences, leading to short-term and long-term complications, tissue damage and organ function deterioration. Some of those complications are irreparable.

Figure 3. SEM reveals single PP fiber degradation, including deep surface cracks. Reduced from ×1,000. Reprinted with permission from Henri Clavé, Department of Gynecologic Surgery, St. George Clinic, Nice, France.

The pathway to clinical complications after mesh is inserted anywhere in the human body is complex. It is difficult to fully separate the effects of the body on the mesh and the effects of the mesh on the body since they are intertwined and develop simultaneously during the postoperative clinical course. Another factor is the skill of the surgeon who implants the mesh. For instance, if the vagina is left too thin or the mesh enters nearby organs, these processes may be accelerated.

Coinciding with the surgical procedure to implant PP mesh, a reaction begins at the insertion site. Bacteria commonly contaminate the mesh by a process that is not modifiable by parenteral or topical antibiotics, especially in the contaminated environment of the vagina. These bacteria produce a slime that encases them and isolates them from body defenses. At the same time there is an immune response in the acute inflammatory phase, followed by the secretion of acid by macrophages, which attacks the mesh and starts the oxidative process already begun by the heat of the manufacturing process. The PP degradation that follows releases additives in the PP that enhance the inflammatory reaction.

All of these processes culminate with the stimulation of fibrosis, leading to mesh shrinkage and chronic pelvic pain if nerve fibers are trapped in the fibrotic reaction. Continued inflammatory responses may lead to erosion or quiescent mesh contamination may be activated, causing overt infection of the PP.

CONCLUSIONS

Based on the available evidence it is clear that PP alters in vivo after implantation. It undergoes vari-

ous processes that lead to degradation, including oxidation, cross-linking, depolymerization and embrittlement. These processes result in various degrees of degradation, and the loss of mechanical and physical properties. PP mesh is not inert. The quest for the perfect vaginal mesh must continue.

REFERENCES

1. Bloch B: International standards for surgical implants. Ann Royal College Surg Eng 1984; **66:** 369.
2. Davis JR: Handbook of Materials for Medical Devices. Materials Park, Ohio: ASM International 2003.
3. Morris PJT: Polymer Pioneers: A Popular History of the Science and Technology of Large Molecules. Philadelphia: Chemical Heritage Foundation 2005; p 76.
4. Usher FC, Cogan JE and Lowry TI: A new technique for the repair of inguinal and incisional hernias. Arch Surg 1960; **81:** 847.
5. Amid PK: Classification of biomaterials and their related complications in abdominal wall hernia surgery. Hernia 1997; **1:** 15.
6. Maier C and Calafut T: Polypropylene. The Definitive User's Guide and Databook. Plastics Design Library. Norwich, New York: William Andrew 1998.
7. Clavé A, Yahi H, Hammou JC et al: Polypropylene as a reinforcement in pelvic surgery is not inert: comparative analysis of 100 explants. Int Urogynecol J 2010; **21:** 261.
8. Serbetci K, Kulacoglu H, Devay AO et al: Effects of resterilization on mechanical properties of polypropylene meshes. Am J Surg 2007; **194:** 375.
9. Tyan YC, Liao JD, Lin SP et al: The study of the sterilization effect of gamma ray irradiation of immobilized collagen polypropylene nonwoven fabric surfaces. J Biomed Mater Res A 2003; **67:** 1033.
10. Culligan P, Heit M, Blackwell L et al: Bacterial colony counts during vaginal surgery. Infect Dis Obstet Gynecol 2003; **11:** 161.
11. King RN and Lyman DJ: Polymers in contact with the body. Environ Health Perspect 1975; **11:** 71.
12. Frostling H, Hoff A, Jacobsson S et al: Analytical, occupational and toxicologic aspects of the degradation products of polypropylene plastics. Scand J Work Environ Health 1984; **10:** 163.
13. Sheftel VO: Toxic Properties of Polymers and Additives. Shawbury, United Kingdom: Rapra Technology 1990.
14. Weiss SJ: Tissue destruction by neutrophils. N Engl J Med 1989; **320:** 365.
15. Williams DF: Review: biodegradation of surgical polymers. J Mater Sci 1982; **17:** 1233.
16. Costello CR, Bachman SL, Ramshaw BJ et al: Materials characterization of explanted polypropylene hernia meshes. J Biomed Mater Res B Appl Biomater 2007; **83:** 44.
17. Schraut W, Wendelgass P, Calzada-Wack JC et al: TNF gene expression in monoctes of low and high responder individuals. Cytokine 1997; **9:** 206.
18. Jongebloed WL and Worst JF: Degradation of polypropylene in the human eye: a SEM-study. Doc Ophthalmol 1986; **64:** 143.
19. Jongebloed WL, Figueras MJ, Humalda D et al: Mechanical and biochemical effects of man-made fibres and metals in the human eye, a SEM-study. Doc Ophthalmol 1986; **61:** 303.
20. Altman AJ, Gorn RA, Craft J et al: The breakdown of polypropylene in the human eye: is it clinically significant? Ann Ophthalmol 1986; **18:** 182.
21. Afonso JS, Jorge RM, Martins PS et al: Structural and thermal properties of polypropylene mesh used in treatment of stress urinary incontinence. Acta Bioeng Biomech 2009; **11:** 27.